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厦门大学

硕士学位论文

PegIFN α 治疗慢乙肝用药指导指标的筛选及NK细胞在慢性乙肝病毒感染中作用的初步探索

PegIFNα treatment of chronic hepatitis B medicationguidance indicators of screening and preliminary investigation of the role of NK cells in patients with chronic hepatitis B virus infection

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摘要

本文针对聚乙二醇干扰素治疗慢乙肝用药指导指标筛选及NK细胞在慢性乙型肝炎感染过程中的作用两个问题进行探讨，共由两部分研究组成。

其中第一部分旨在筛选能够指导聚乙二醇干扰素治疗慢性乙型肝炎用药的指标。乙型肝炎病毒（HBV）感染是我国乃至全世界最重要的公共卫生问题之一，严重威胁人类健康。目前用于乙肝病毒感染的治疗药物主要有两类：干扰素（普通干扰素（IFNα）和聚乙二醇干扰素（PegIFNα））和核酸类似物（拉米夫定（Lamivudine,LAM）、替比夫定（Telbivudine,LdT）等）。两类药物中因干扰素具有直接抗病毒和免疫调节双重作用，所以临床抗病毒治疗中应用较为广泛，其具有无耐药、有免疫介导的控制HBV感染的潜在作用的优点，从而使治疗结束时患者有机会得到较高的HBeAg血清学转换、更持久的病毒学应答以及HBsAg清除，使这些患者可维持HBV DNA在检测下限。然而使用干扰素α常规治疗6个月治疗的效应率为25%-40%，复发率和停药后的后续效应率均约为15%~20%，持续效应率也仅为25%~40%。治疗效应有很大的个体差异，30%以上的病例需要6个月以上的治疗。另外从治疗费用上看，干扰素α与其他药物有很大区别，治疗1年的大致费用PegIFNα-2a/2b远高于LAM、ADV和ETV等药物。如慢乙肝患者使用干扰素α进行6个月以上乃至24月的抗病毒治疗，治疗失败不仅给患者带来身体和精神上痛苦，而且要承受巨大的经济压力。现在虽然有一些指标用于干扰素α应答的预测，但并不能满足临床需要。有鉴于此，本研究首先通过收集聚乙二醇干扰素治疗前及治疗后24周的慢性乙肝患者的血清样本，以病毒学应答和非病毒学应答进行分组，使用luminex 200检测46种人细胞因子，所有细胞因子中CXCL9和IP-10基线水平病毒学应答组高于非应答组，应答组在治疗过程中下降然而非应答组变化无统计学意义。MIP-1d在病毒学应答组基线水平和治疗过程中均显著高于非应答组，而TARC则在毒学应答组基线水平和治疗过程中均显著低于于非应答组。基线水平HBV DNA 、HB eAg、TARC、MIP1d、CXCL9、CXCL6和IP10具有较好的干扰素治疗病毒学应答的预测效果，其受试者工作特征曲线显示线下面积分别为0.879、0.964、0.77、0.787、0.799、0.722和0.787（p=0.001、p<0.001、p=0.021、p=0.01、p=0.01、p=0.05和p=0.013）。另外CXCL9、CXCL6与ALT具

有显著相关性（r= 0.588、p=0.002；r=0.530、p=0.005）。综上所述,本研究发现在慢性乙型肝炎治疗前及治疗过程中细胞因子的水平对干扰素治疗慢性乙肝的病毒学应答具有预测效果，可作为慢性乙肝治疗前选择用药的指标。

第二部分旨在初步探索NK细胞在慢性乙肝病毒感染过程中对机体抗病毒免疫反应的影响机制。慢性HBV感染患者机体缺乏有效的抗病毒免疫反应，宿主无法及时有效清除体内病毒，致使病毒持续感染。这种免疫耐受状态可能是由于宿主抗HBV的细胞免疫应答处于无能或被抑制状态造成的。新近研究发现慢性病毒感染者免疫耐受状态的形成可能与NK细胞活化后抑制T细胞反应密切相关，NK细胞通过不同途径限制T细胞功能，包括NK细胞通过影响抗原递呈影响T细胞功能,NK细胞通过分泌细胞因子改变T细胞反应，或通过NK的裂解作用调节T细胞免疫反应等。通过阻断NK活性或直接删除NK细胞可能恢复病毒特异性T细胞活性，逆转免疫耐受，激活宿主抗病毒免疫应答以促进HBV的清除，可能是治疗慢乙肝药物的研究方向之一。本研究从在体和体外实验两个方面去初步探索NK细胞在慢乙肝中的作用机制。在体实验中，我们利用HBV转基因小鼠为模型，使用Anti-NK1.1单抗阻断NK细胞活性，结果显示阻断NK细胞活性后可使HBV转基因小鼠血清HBV DNA和HBsAg水平发生显著降低，在阻断后的第三天效果最为显著，HBV DNA较治疗前下降约1.5log10，HBsAg较治疗前下降约1.0log10。体外实验中，提取慢乙肝患者PBMC后分选删除NK后使用核心抗原多肽与PBMC及删除NK细胞的PBMC共培养，结果显示NK删除后大部分患者能恢复病毒特异性CD8+IFN-γ+T细胞的比例。综上所述，本研究在HBV转基因小鼠模型和体外实验中初步证实了NK细胞的删除可恢复宿主的抗病毒状态，为NK细胞作为慢乙肝免疫治疗新靶向奠定了基础。

关键词：乙型肝炎病毒，干扰素α,细胞因子，自然杀伤细胞。

Abstract

In this thesis, we focused on the screening index of Polyethylene glycol (PEG) interferon treatment of chronic hepatitis B and the role of NK cells acted in chronic hepatitis B infection. This thesis consists of two sections.

In the first section, we were trying to screen the index which could guidethe PEG interferon treatment of chronic hepatitis B. Hepatitis B virus (HBV) infection is one of the most critical public health issues which threatened the human health in our country, even in the world. Recent therapy methods could be divided into two categories, interferon (IFNα, PEGIFNα) and nucleotide analogs (lamivudine、Telbivudine, etc.). Due to thedual role (direct antiviral and immune regulation) interferon actedin the therapy process, it has been widely applied in clinical antiviral treatment. The interferon has numerous advantages including no drug resistance, immune-mediated control HBV infection. All of these features offered more opportunities to obtain higher HBeAg seroconversion, longer virological response and better HBsAg clearance, which could help the patients maintaining the HBV DNA content under the detection limit. However, the treatment effect rate of interferon-α based conventional method is ranging from 25% to 40%, the recurrence rate and subsequent effect after drug withdrawal rate is ranging from 15% to20% and the persistent effect rate is only 25% ~ 40%. Moreover, the treatment effect was largely related to the individual difference, more than 30% of the cases requirelong time treatment, generally, longer than six months. In addition, from the aspects of treatment costs, interferon-α is very different from other drugs, the average cost of PEGIFNα-2a/2b is much higher than that of LAM, ADV and ETV drugs. For instance, chronic hepatitis B patients utilize interferon-α based therapyfor more than six months, even twenty four months antiviral treatment, the failure of treatment will cause not only physical and mental suffering, but also a tremendous economic pressure. Though there are some indicators used for interferon-αresponse prediction,

it does not meet the clinical needs. To address this issue, we firstly gathered the serum samples from chronic hepatitis B patients before and after twenty four weeks polyethylene glycol interferon treatment. Then, categorizing them into two groups based on the virologic and non-virological response. Utilizing luminex 200 detectsforty six kinds of human cytokines, all the samples'CXCL9 and IP-10 baseline virological response group higher than the non-virological response group and virological group fell in the process of treatment,whereas non-virological response group was no significant change.Virological response group compared with the non-virological response, MIP-1d at baseline and during treatment were significantly higher than non-virological response, while TARC at baseline and during treatment were significantly lower than in non-virological response.HBV DNA, HB eAg, TARC, MIP1d, CXCL9, CXCL6 and IP10 baseline level has an expected effect for interferon treatment. The AUROC value of HBV DNA, HB eAg, TARC, MIP1d, CXCL9, CXCL6 and IP10 were 0.879,0.964,0.77,0.787,0.799,0.722 and 0.787 ($p = 0.001$, $p < 0.001$, $p = 0.021$, $p = 0.01$, $p = 0.01$, $p = 0.05$ and $p = 0.013$). Additionally, CXCL9, CXCL6 and ALT have obvious correlation ($r = 0.588$, $p = 0.002$； $r = 0.530$, $p = 0.005$). In summary, we found that cytokines level of pre-treatment and in-treatment has the potential to select chronic hepatitis B patients who can respond to PEG-IFN. Therefore, cytokines can be used as an index for former choice drug therapy of chronic hepatitis B.

The second section was an investigationabout the antiviral immunity reaction mechanism of NK cells in the process of chronic hepatitis b virus infection. Patients with chronic HBV infection are lacking of effective antiviral immunity reaction.The host cannot remove the virustimely and effectively, resulting in persistent infection. This immune tolerance state may due to the host anti HBV cellular immune responses in a state of incompetence or suppressed. Latest research has shown that the activation of NK cells suppresses T cell responses is responsible for this immune tolerance state formation in chronic HBV infection. NK cells use different ways to limit T cell function, including antigen affecting induced function change, cytokine secretion alteredreaction, lytic activity mediated immunity reaction and etc.

Depletion of NK cellsorNK-deficient mice demonstrates that NK cells have an intrinsic ability to inhibit adaptive T cell responses against virus，it becomes one of the most promising directions for developing new immunotherapy drugs of chronic HBV. We investigated the mechanism of the role which NK cells acted in chronic hepatitis B both in vivo and in vitro. Selective depletion of NK cells using 25 μg anti-NK1.1 monoclonal antibodies in HBV transfecting mice resulted in serum HBV DNA and HbsAg levels decreased significantly, the most significant change occurs in the deletion of NK cells at the third day. Comparing with pre-injection, in serum HBV DNA decreased1.5log10, and HBsAg decreased 1.0log10.In vitro, first of all, extracting PBMC from patients with chronic hepatitis B and sorting delete NK cells, followed by using the core antigen peptide libraries co-cultured with PBMC and NK cells deleted PBMC. The results showed that the majority of patients can recover virus-specific cell proportional of CD8 +IFN-γ + T cells after NK deletion. To sum up, we have preliminary confirmed that the deletion of NK cells could restore the host state of antiviral and laid a foundation for chronic hepatitis B target as a novel immunotherapy.

Key words:HBV ,IFN α ,cytokines , NK cell.